TOPIC 03-2 – Oxydative stress, NO, aging

May 13th, Friday 2011

0316

Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a "mitohormesis" mechanism involving reactive oxygen species and PGC-1

Jamal Bouitbir [Orateur] (1), Anne-Laures Charles (1), Andoni Echaniz-Laguna (2), Michel Kindo (3), Frédéric Daussin (1), Johan Auwerx (4), François Piquard (1), Bernard Geny (1), Joffrey Zoll (1)
(1) Hôpital Civil, Service de physiologie et explorations fonctionnelles, Strasbourg, France – (2) Nouvel Hôpital Civil, Service de neurologie, Strasbourg, France – (3) Nouvel hôpital civil, Service de chirurgie cardiaque, Strasbourg, France – (4) Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Suisse

Aims: Statins protect against cardiovascular-related mortality but induce skeletal muscle toxicity. To investigate mechanisms of statins, we tested the hypothesis that statins optimized cardiac mitochondrial function but impaired vulnerable skeletal muscle by inducing different level of reactive oxygen species (ROS).

Methods and results: In atrium of patients treated with statins, ROS production was decreased and oxidative capacities were enhanced together with an extensive augmentation of mRNAs expression of PGC-1 family. However, in deltoid biopsies from patients with statin-induced muscular myopathy, oxidative capacities were decreased together with ROS increase and a collapse of PGC-1 mRNA expression. Several animal and cell culture experiments were conducted and showed by using ROS scavengers that ROS production was the triggering factor responsible of atorvastatin-induced activation of mitochondrial biogenesis pathway as well as improvement of antioxidant capacities. Conversely, in skeletal muscle, the large ROS production augmentation following treatment induced mitochondrial impairments, and reduced mitochondrial biogenesis mechanisms. Quercetin, an antioxidant molecule, was able to counteract skeletal muscle deleterious effects of atorvastatin in rat.

Conclusion: Statins acted *via* a "mitohormesis mechanism": when they induced low ROS production as in cardiac muscle, they activated mitochondrial biogenesis and protected them, whereas when they highly increased ROS production as in glycolytic skeletal muscle, they impaired muscular oxidative capacities, and deactivated mitochondrial biogenesis. These findings identify statins as a new activating factor of cardiac mitochondrial biogenesis and showed importance of ROS/PGC-1 signalling pathway as a key element in regulation of muscular mitochondrial function.

0140

Genetic deletion of protein tyrosine phosphatase 1B protects against endotoxinic shock and improves endothelial dysfunction

David Coquerel [Orateur], Elodie Gomez, Sylvanie Renet, Brigitte Dautreau, Vincent Richard, Christian Thuillez, Fabienne Tamion
INSERM U644, Rouen, France

Sepsis is one of the major causes of mortality in critically ill patients. Endothelial dysfunction (ED) and especially impaired NO production by endothelial NO synthase (eNOS) plays a crucial role in the pathogenesis of sepsis, several studies showed that increased endothelial NO production improves survival in sepsis. Our laboratory has shown that the protein tyrosine phosphatase 1B (PTP1B) negatively modulates NO production and that its inhibition may reduce ED. The aim of the present study is to assess the effect of genetic deletion of PTP1B (PTP1B-/-) on both inflammatory state and endothelial function in mice with endotoxinic shock induced by lipopolysaccharid (LPS) intraperitoneal injection followed by a subcutaneous fluid resuscitation. Vascular function on mesenteric arteries was evaluated in vitro on arteriograph system and plasma was collected to measure the inflammatory cytokine (TNF-a, IL1-b) by Elisa multiplex assay, 4 hours (H4) and 8 hours (H8) after LPS injection.

Compared to WT, PTP1B-/- mice showed a marked decrease in mortality (30% vs 100%, n=20). In WT mice, LPS induce a severe ED as shown by a decreased in acetylcholine induced dilatation (WT 92±1% vs WT H8 42±5%, p<0,001) which was improved in PTP1B-/- mice (56%). Moreover insulin induced dilatation was not altered by sepsis but was higher in PTP1B-/- mice compared to WT mice (PTP1B-/- 45±8% vs WT 29±3%, p=0,039). The improvment of ED was associated with decreased circulation NO levels and an enhancement of heme oxygenase-1 expression. However the plasma levels of IL1-b and TNF-a were significantly higher in PTP1B-/- mice than WT mice at H4 demonstrating a role of PTP1B in the inflammatory process.

In conclusion, genetic deficiency of PTP1B confers a resistance against LPS induced sepsis despite in increased inflammatory state. The inhibition of PTP1B could be a new treatment of the endothelial dysfunction associated with sepsis.

0427

Regulation of Semicarbazide-sensitive amine oxidase in adipocytes under hypoxic conditions: mechanisms and pathophysiological implications

Xavier Repesse (1), Bruno Feve [Orateur] (2), Gérard Chétrite (3), Jacques Duranteau (2), Adeline Muscat (3)
(1) APHP-Hôpital de la Pitié Salpétrière, Réanimation Médicale, Paris, France – (2) APHP-INSERM, Service d'Endocrinologie, Paris, France – (3) INSERM, INSERM U938, équipe 'maladies du tissu adipeux', Paris, France

«Semicarbazide-sensitive amine oxidase» (SSAO) is highly expressed in plasma membranes of adipocytes and vascular smooth muscle cells. It metabolizes primary amines as substrates to generate the corresponding aldehyde, H_2O_2 and ammonia. It has been suggested that it might be involved in endothelial injury, but also in cellular glucose uptake. The aim of this study was to test whether SSAO expression and activity were modulated under hypoxic conditions frequently encountered either in intensive care units patients, or in adipose tissue of obese individuals. We show that in murine 3T3-L1 adipocytes and human adipose tissue explants, physical or chemically-induced (caused by cobalt chloride or deferoxamine) hypoxia strongly reduces SSAO expression and activity. This effect was dose-and time-dependent, reversible, and unrelated to impairment of cell viability. The regulation of SSAO by hypoxia does not implicate the transcription factor HIF-1-alpha or oxidative stress phenomena. By contrast, it involves a change in the adipocyte acid-base balance. The negative modulation of SSAO under hypoxic conditions may represent an adaptive mechanism limiting the production of toxic molecules such as aldehydes and H_2O_2, and protecting these territories under hypoxic conditions.

0113

Chronic inhibition of protein tyrosine phosphatase 1B improves diastolic function and limits endothelial dysfunction in insulin resistant / obese mice

Elodie Gomez [Orateur] (1), Najah Harouki (1), Paul Mulder (1), Fabrice Bauer (1), Rob Hooft (2), Christian Thuillez (1), Vincent Richard (1)
(1) Inserm U644, Rouen, France – (2) Merck-Serono, Geneve, France

Insulin resistance and obesity induce both endothelial and cardiac dysfunction. Insulin induces endothelial release of NO, and this pathway is altered in insulin resistance. Protein tyrosine phosphatase 1B (PTP1B) modulates insulin action, but we have revealed that it also negatively regulates NO production, and that PTP1B inhibitors improve endothelial function in heart failure. This study assessed the effect of PTP1B inhibition on cardiac and endothelial function in insulin-resistant mice.

Mice were fed with normal or high fat (60%) diet (HFD) for 4 months. HFD mice were either untreated or treated for 3 months with a PTP1B inhibitor, starting after 1 month HFD. Cardiac function was assessed by echography, tissue Doppler imaging (TDI) and left ventricular pressure volume relationships, while endothelial function of coronary and mesenteric arteries was evaluated *in vitro* in a small vessel myograph.

HFD induced insulin resistance (blood glucose 120 min after insulin: ctl 1.9±0.4; HFD 5.8±0.7 mmol/L; n=11; p<0.001), and increased body weight (ctl 28±1; HFD 36±1g; n=11; p<0.01).

TDI showed that HFD decreased Ea/Aa ratio (ctl 1.58±0.08 n=6; HFD 1.24±0.07 n=5; p<0.05) and increased end diastolic pressure-volume relationship (EDPVR: ctl 1.19±0.26 n=5; HFD 4.85±1.17 n=7, p<0.05), demonstrating diastolic dysfunction, in the absence of changes in parameters of systolic function. This diastolic dysfunction was reduced by PTP1B inhibition (Ea/Aa ratio: 1.39±0.06; EDPVR: 1.38±0.31 n=6; p<0.05).

HFD impaired insulin-induced relaxations of mesenteric (control 62±5 n=11; HFD 32±6% n=10; p<0.01) and coronary arteries (ctl 91±1 n=8; HFD 29±10 n=11; p<0.01). This impairment was reduced by PTP1B inhibition (mesenteric: 60±5 n=10; coronary: 91±1 n=9; p<0.01).

Thus, chronic PTP1B inhibition improves diastolic dysfunction and restores endothelial function, suggesting that it may be a treatment of cardiovascular complications in diseases associated with insulin resistance.

0279

Crataegus special extract WS1442 prevents aging-related COX-mediated endothelium-dependent contractions

Noureddine Idris Khodja [Orateur] (1), Cyril Auger (1), Egon Kokh (2), Valérie Schini-Kerth (1)
(1) CNRS UMR 7213, Faculté de pharmacie. Université de Strasbourg, Illkirch-Graffenstaden, France – (2) GmBH & Co. KG, Karlsruhe, Allemagne

The present study has evaluated whether the *Crataegus* (*Hawthorn species*) special extract WS®1442 prevents the development of aging-related endothelial dysfunction in rats, and, if so, to determine the underlying mechanisms. Wistar rats received either a control diet or the same diet containing 100 or 300 mg/kg/day of WS®1442 from week 25 until week 65. Vascular reactivity was assessed in the mesenteric artery using organ chambers, oxidative stress by dihydroethidine staining and cyclooxygenase-1 (COX-1) and -2 (COX-2) expression by immunohistochemistry. Acetylcholine-induced endothelium-dependent relaxations were blunted in artery rings of 1-year old compared to young rats (16 weeks). The endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation was reduced whereas the nitric oxide (NO) component was not affected. Endothelial dysfunction was also associated with endothelium-dependent contractile responses to acetylcholine in the presence of inhibitors of NO and EDHF-mediated responses. Both endothelium-dependent relaxations and induction of contractile responses in 1-year old rats were normalized by indomethacin, COX-1 and COX-2 inhibitors and a TP receptor antagonist. WS®1442 treatment prevented aging-related endothelial dysfunction. Vascular oxidative stress and an upregulation of COX-1 and COX-2 were observed in 1-year old rats compared to young rats; WS®1442 prevented both of these effects. Thus, aging is associated with an endothelial dysfunction in the mesenteric artery of 1-year old rats involving a reduced EDHF-mediated relaxation and the induction of COX-dependent contractile responses. Chronic intake of the WS®1442 prevented aging–related endothelial dysfunction by reducing the EDCF-mediated contractile responses most likely by normalizing the expression of COX-1 and COX-2.

0093

Effects of Hydrogen Sulfide in a rat model of Ischemia Reperfusion

Khodr Issa [Orateur]
Groupe choc – contrat avenir Insem, Vandoeuvre Les Nancy, France

Hydrogen sulfide (H_2S) is qualified as the third gasotransmitter beside nitric oxide (NO) and carbon monoxide (CO). The location of the H_2S synthesizing enzymes as well as the detection of endogenous levels of H_2S in the tissues suggest that the cardiovascular system is a source of H_2S. H2S relaxes vascular smooth muscle both in vitro and in vivo probably by opening K^+_{ATP} channels. The analysis of the literature shows that according to the circumstances and the studied model, H_2S or the inhibitors of its synthesis improve the hemorrhagic shock induced hemodynamic disturbances. In this work, we characterized the effects of H_2S or its inhibition on hemodynamic and tissue metabolism in a model of ischemia-reperfusion induced by hemorrhagic shock. Our assumption was that H_2S injected at the time of the reperfusion could decrease the consequences of shock and reperfusion in decreasing NO and oxygen free radicals production. Thus, we developed a model of ischemia-reperfusion induced by hemorrhagic shock and we studied the effects of H_2S and of an inhibitor of its synthesis (PAG) on the hemodynamic, lactate concentration, tissue NO, plasma nitrite/nitrate and vascular/myocardial mRNA expression.

We found that 1) an intravenous bolus of NaHS an injectable form of H_2S, infused 10 minutes before the blood retransfusion, limited the decrease in arterial pressure induced by shock and decreased plasmatic lactate, a witness of tissue suffering, 2) this hemodynamic improvement was associated with a fall in myocardial iNOS mRNA expression, a reduction in the concentration of plasmatic NOx and a reduction of aortic and myocardial concentrations of NO and superoxide anion,3) the inhibition of H_2S with PAG worsened hemodynamics and tissue consequences of shock, 4) the in vivo use of a selective inhibitor of vascular potassium channels improved the hemodynamic answer of the treated rats, and this improvement was even more important in the presence of NaHS.

On the whole, our results suggest a beneficial effect of a single bolus of NaHS to limit the noxious effects of ischaemia reperfusion. NaHS seems to act by limiting the production of NO and anion superoxyde.

0159

Abnormal microvascular reactivity to local cooling in primary Raynaud's phenomenon

Matthieu Roustit [Orateur], Sophie Blaise, Claire Millet, Jean-Luc Cracowski
CHU de Grenoble, Centre d'Investigation Clinique – INSERM, Grenoble Cedex 09, France

Objective: Raynaud's phenomenon (RP) is defined as episodic ischemia of the extremities in response to cold. Although the structure of skin capillaries is normal in primary RP, some data suggest impairment of microvascular function. The primary objective of this study was to assess whether microvascular reactivity to local cooling was impaired in RP. We further evaluated the contribution of sensory nerves.

Methods: We recruited 21 patients with primary RP and 20 healthy volunteers matched on age and gender. After a 10-min baseline at 33°C, skin temperature was cooled at 15°C during 30 min on the forearm and the finger of each participant while monitoring perfusion with a custom-design laser Doppler flowmetry cooling probe. Perfusion was also assessed after local anesthesia to test whether neurovascular response was involved in the initial vasoconstriction.

Results: Microvascular reactivity on the finger was exaggerated in RP patients compared to controls (AUC0-30 were -106237.2 and -69544.3%BL.s-1, respectively; p=0.03) whereas we observed no significant difference on the forearm (AUC0-30 were -57183.5 and -55472.9%BL.s-1, respectively; p=0.78). Local anesthesia increased skin blood flow in patients with RP (AUC0-15 increased from -51415.2 to -37486.1%BL.s-1; p=0.05) whereas it did not affect reactivity in controls (p=0.88).

Conclusion: Our study shows with a non-invasive test that microvascular reactivity to local cooling is impaired on the dorsum of the finger in primary RP. The initial transient vasodilation was blunted on the dorsum of the finger, and that part of this abnormal response in primary RP depends on cold sensory nerves.

0161

Oral sildenafil increases sodium nitroprusside iontophoresis induced skin hyperaemia in healthy volunteers

Sophie Blaise, Marcin Hellmann, Matthieu Roustit [Orateur], Claire Millet, Jean-Luc Cracowski
CHU de Grenoble, Centre d'Investigation Clinique – Inserm, Grenoble Cedex 09, France

Objective: Sildenafil, a specific inhibitor of phosphodiesterase 5A (PDE5A), is currently tested as a treatment for severe Raynaud's phenomenon. Here we tested whether sildenafil, alone or combined with local sodium nitroprusside (SNP) delivered through skin iontophoresis, increased forearm cutaneous blood conductance in healthy volunteers, and to assess how well this combination was tolerated.

Design and method: Ten healthy volunteers were enrolled. Variations in cutaneous vascular conductance following oral administration of 50 mg or

100 mg of sildenafil with or without SNP iontophoresis were expressed as a percentage of maximal cutaneous vascular conductance and were monitored using laser Doppler imaging. SNP iontophoresis was performed on the ventral surface of the forearm, one hour after application of lidocaine/prilocaine cream.

Results: Sildenafil at 100 mg, but not 50 mg, increased overall responses (area under the curve) (44%) and peak responses (29%) to SNP iontophoresis. Sildenafil at 100 mg, but not 50 mg, increased baseline cutaneous vascular conductance (75%). Incidence of headache was not changed when SNP iontophoresis was combined with sildenafil. One episode of symptomatic arterial hypotension occurred in a volunteer given 50 mg sildenafil, 30 min after the beginning of SNP iontophoresis.

Conclusions: Oral sildenafil at 100mg potentiated local skin hyperaemia induced by SNP iontophoresis, with no increased incidence of headaches. The combination of oral specific PDE5A inhibitor and nitrates administered through skin iontophoresis deserve further investigation in diseases such as severe Raynaud's phenomenon, with particular attention to the incidence of arterial hypotension.

0163

Cathodal iontophoresis of treprostinil and iloprost induce a sustained increase in cutaneous flux in rat

Sophie Blaise (1), Matthieu Roustit [Orateur] (1), Claire Millet (1), Christophe Ribuot (2), Jean Boutonnat (2), Jean-Luc Cracowski (1)

(1) CHU de Grenoble, Centre d'Investigation Clinique – INSERM, Grenoble Cedex 09, France – (2) Université Joseph Fourier, INSERM U1042, Grenoble, France

Background: The treatment of scleroderma-related digital ulcers is still a therapeutic challenge. The most effective drugs are prostacycline analogues. However, their usage is limited to an intravenous route of administration and by their frequent side effects. The objective of this study was to test whether treprostinil, iloprost and epoprostenol can induce sustained vasodilatation in rats when delivered locally using cutaneous iontophoresis.

Methods: Treprostinil, iloprost and epoprostenol were delivered by cathodal and anodal iontophoresis onto the hindquarters of anaesthesized rats (n=8 for each group). Skin blood flow was quantified using laser Doppler imaging and cutaneous tolerance was assessed from day 0 to day 3.

Results: Cathodal but not anodal iontophoresis of treprostinil (6.4 mM), iloprost (0.2 mM) and epoprostenol (1.4 mM) induced a significant and sustained increase in cutaneous blood flow. The effects of treprostinil and iloprost were significantly different from those of treprostinil vehicle. Only weak effects were observed when both drugs were applied locally without current. Skin resistance was unchanged in areas treated with prostacyclin analogues. Finally, skin tolerance was good, with no evidence of epidermal damage.

Conclusions: Cathodal iontophoresis of treprostinil and iloprost increases cutaneous blood flow with a good local tolerance. The effects of cathodal iontophoresis of these drugs should be investigated in humans, as they could have potential as new local therapies for digital ulcers in patients with scleroderma.